Thyroid hormone transporters—functions and clinical implications

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Abstract | The cellular influx and efflux of thyroid hormones are facilitated by transmembrane protein transporters. Of these transporters, monocarboxylate transporter 8 (MCT8) is the only one specific for the transport of thyroid hormones and some of their derivatives. Mutations in *SLC16A2*, the gene that encodes MCT8, lead to an X-linked syndrome with severe neurological impairment and altered concentrations of thyroid hormones. Histopathological analysis of brain tissue from patients who have impaired MCT8 function indicates that brain lesions start prenatally, and are most probably the result of cerebral hypothyroidism. A *Slc16a2* knockout mouse model has revealed that Mct8 is an important mediator of thyroid hormone transport, especially T_3, through the blood–brain barrier. However, unlike humans with an MCT8 deficiency, these mice do not have neurological impairment. One explanation for this discrepancy could be differences in expression of the T_4 transporter OATP1C1 in the blood–brain barrier; OATP1C1 is more abundant in rodents than in primates and permits the passage of T_4 in the absence of T_3 transport, thus preventing full cerebral hypothyroidism. In this Review, we discuss the relevance of thyroid hormone transporters in health and disease, with a particular focus on the pathophysiology of MCT8 mutations.

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Introduction

The thyroid hormones, T_4 (3,5,3',5'tetraiodo-L-thyronine) and T_3 (3,5,3'tri-iodo-L-thyronine; also known as tri-iodothyronine) are iodinated amino acids produced and secreted by the thyroid gland. These hormones regulate many developmental and metabolic processes. The nuclear T_3 receptors are ligand-modulated transcription factors encoded by two genes, *THRA* and *THRB*. These genes encode several receptor proteins, of which three (thyroid hormone receptor α1, thyroid hormone receptor β1 and thyroid hormone receptor β2) interact with T_3, which results in tissue-specific and developmentally-dependent transcriptomic changes.[1] In the developing cerebral cortex, 500–1,000 genes are directly or indirectly affected by thyroid hormones.[2] In addition, both T_4 and T_3 perform nongenomic, extranuclear actions. For example, T_3 might interact with a plasma-membrane-associated thyroid hormone receptor α variant,[3] and with cytoplasmic thyroid hormone receptor β,[4] while T_4 interacts with integrin $\alpha_v\beta_3$ and activates diverse signalling pathways such as the phosphoinositide 3-kinase pathway and mitogen-activated protein kinase pathways.[5,6]

Metabolism of thyroid hormones includes the processes of deiodination, deamination, decarboxylation, sulphation and glucuronidation, which have been extensively reviewed elsewhere.[7] The most relevant pathway for the discussion in this Review is deiodination, a process that activates or inactivates thyroid hormones. Deiodinases are selenoproteins that catalyze the removal of specific iodine atoms from the phenolic or tyrosyl rings of the iodothyronine molecule. Type 1 iodothyronine deiodinase and type 2 iodothyronine deiodinase (DIO1 and DIO2, encoded by the *DIO1* and *DIO2* genes, respectively) have phenolic, or 'outer' ring, activity and convert T_4 to T_3.[8] In extrathyroidal tissues, this pathway generates ~80% of the total body pool of T_3.[9] Type 3 iodothyronine deiodinase (DIO3, encoded by the *DIO3* gene) and DIO1 have tyrosyl, or 'inner' ring, activity and convert T_4 and T_3 to the inactive metabolites 3,3'5'-triiodo-L-thyronine (rT_3) and 3,3'-diiodo-L-thyronine (T_2), respectively; rT_3 is then further metabolized by DIO1 to T_2.

Until the 1970s, the passage of thyroid hormones through the cell membrane was thought to be a process of passive diffusion.[10] However, for uncharged molecules, the basal permeability of membranes decreases rapidly with size, and is low for molecules with a molecular weight >100.[11] A number of investigators described the presence of low affinity transport systems for thyroid hormones in tissues and cultured cells.[10] However, not until 2004,[12,13] when mutations in a gene encoding a previously identified cell membrane transporter for thyroid hormone were identified, was monocarboxylate transporter 8 (MCT8)[14,15] established as having pathophysiological relevance of thyroid hormone transport.

Several proteins with overlapping specificity have the capacity to transport thyroid hormones across cell membranes, including the monocarboxylate transporters (MCT), organic anion transporter polypeptides (OATP), large neutral amino acid transporters (LAT)

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Key points

- Many proteins can mediate thyroid hormone transport, but only mutations in genes encoding MCT8, MCT10 and OATP1C1 have pathophysiological effects attributed to this process
- MCT8 mutations lead to Allan–Herndon–Dudley syndrome, which is characterized by truncal hypotonia and results in spastic quadriplegia, lack of speech, severe intellectual deficit and altered thyroid hormone concentrations
- MCT8 deficiency impairs the transfer of thyroid hormones across the blood–brain barrier
- Mct8-deficient mice lack neurological impairment possibly due to the presence of Oatp1c1, a T_4 transporter, but levels of OATP1C1 in the primate blood–brain barrier are very low
- Histopathological studies of patients with mutations in MCT8 support the concept that defective thyroid hormone action in the brain during development leads to the neurological syndrome

Table 1 | Substrates and K_m of thyroid hormone transporters

Protein	T_3 (μM)	T_4 (μM)	rT_3 (μM)	Transport	Species	Reference
Mct8	4.0	4.70	2.2	T_3, T_4, rT_3	Rat	Friesema *et al.*[15]
MCT10	ND	ND	ND	T_3, T_4	Human	Friesema *et al.*[23]
Oatp1c1	ND	0.18	ND	T_3, T_4, rT_3	Rat	Sugiyama *et al.*[43]
Oatp1c1	ND	0.34	0.46	T_3, T_4, rT_3	Mouse	Tohyama *et al.*[115]
OATP1C1	ND	0.09	0.13	T_3, T_4, rT_3	Human	Pizzagalli *et al.*[42]
Oatp1a4	5.9	6.50	ND	T_3, T_4	Rat	Abe *et al.*[41]
OATP3A1	ND	ND	ND	T_4	Human	Huber *et al.*[44]
Oatp2b1	ND	0.77	ND	T_4	Rat	Leuthold *et al.*[45]
OATP4A1	0.9	ND	ND	T_3, T_4, rT_3	Human	Fujiwara *et al.*[116]
Lat1	0.8	7.90	12.5	T_3, T_4, rT_3	Mouse	Ritchie & Taylor[117]
LAT2	(<LAT1)	(<LAT1)	ND	T_3, T_4, rT_3	Human	Friesema *et al.*[32]
NTCP	ND	ND	ND	T_4s, T_3s	Human	Visser *et al.*[30]

Abbreviations: K_m, Michaelis constant; LAT/Lat, large neutral amino acid transporters; MCT/Mct, monocarboxylate transporters; ND, not determined; NTCP, sodium/taurocholate cotransporting polypeptide; OATP/Oatp; organic anion transporter polypeptides; T_3s, T_3 sulphate; T_4s, T_4 sulphate; rT_3, 3,3′5′-triiodo-L-thyronine.

and the sodium/taurocholate cotransporting polypeptide (SLC10A1 commonly known as NTCP). The transport kinetics and specificity for thyroid hormones and their related compounds have been thoroughly reviewed elsewhere.[16] Briefly, the Michaelis constant (K_m) for T_3 and T_4 transport is usually in the μM range (Table 1), whereas the total concentration of thyroid hormones in tissues is 3–30 nM for T_4 and 1.5–8.0 nM for T_3.[17] Owing to extensive binding to serum and cellular proteins, the combined concentration of free T_3 and T_4 is 10–30 pM.[18,19] Most transporters, with the notable exception of MCT8, are carriers for other substances, such as amino acids or steroids, which have tissue concentrations far higher than thyroid hormones. Consequently, we do not believe that one can make any conclusions on the physiological significance of most transporters solely from their *in vitro* transport kinetics. However, the identification of pathogenic mutations and results from animal studies of transporter inactivation indicate that only MCT8, MCT10 and solute carrier organic anion transporter family member 1C1 (commonly known as OATP1C1) have a pathophysiological role in thyroid hormone transport.

Advances in our understanding of the mechanisms underlying diseases mediated by defects in thyroid hormone transport and the molecular genetics of mutations in the MCT8 protein have stimulated studies on the physiological roles of other transporter proteins considered as 'secondary' thyroid hormone transporters.[20] In this Review, we describe the different families of thyroid hormone transporters, their physiological relevance in thyroid hormone transport and their clinical implications. Given the importance of thyroid hormone transport for the brain, in this Review we will pay special attention to the transporter physiopathology affecting this organ.

Thyroid hormone transporter families

The terminology describing thyroid hormone transporter proteins is complicated and confusing, with many individual proteins having different names (Table 2). Most thyroid hormone transporter proteins are widely expressed among tissues.[16,20] An example of the distribution of thyroid hormone transporters in the mouse brain is shown in Figure 1. These data have been extracted from a transcriptomic analysis of the developing mouse cerebral cortex,[21] and can be used to formulate hypotheses on the specific role of individual proteins on thyroid hormone transport in the developing brain.

MCT

MCTs are proteins with 12 transmembrane domains that transport monocarboxylates such as pyruvate, lactate, ketone bodies, carnitine and aromatic amino acids.[22] Two members of this family function as sodium and proton-independent thyroid hormone transporters:[22] MCT8 (encoded by *SLC16A2*) and MCT10 (encoded by *SLC16A10*), which mediate the transport of thyroid hormones through the plasma membrane.[23] MCT8 is highly specific for T_4 and T_3 transport (Table 1), but also transports rT_3 and T_2.[15] To date, MCT8 is the only thyroid transporter with known pathogenic mutations in humans.[12,13] In the developing mouse brain, *Slc16a2* is expressed in astrocytes, neurons, oligodendrocyte precursor cells and endothelial cells (Figure 1).[24–26] MCT10 is a transporter of aromatic amino acids (such as, phenylalanine, tyrosine and tryptophan) and also transports T_3 and T_4. Compared with MCT8, MCT10 is slightly more efficient at transporting T_3, and less efficient in T_4 transport.[23] In the developing mouse brain, *Slc16a10* is expressed prominently in microglia (Figure 1),[27] and is expressed in neurons and white matter cells in late postnatal and adult mice.[28]

Liver sodium/taurocholate cotransporter

Liver sodium/taurocholate cotransporter, commonly known as NTCP, is a seven transmembrane domain glycoprotein that is involved in the enterohepatic circulation of bile acids and is a member of the solute carrier gene family 10A.[29] This protein (encoded by *SLC10A1*) transports the sulphate derivatives of T_4 and T_3,[30] and might also transport these derivatives into the liver for deiodination.[30]

Table 2 | Nomenclature of thyroid hormone transporters

Gene	Old gene name	Protein	Alternative protein names	Organism
SLC16A2/Slc16a2	None	MCT8/Mct8	XPCT/Xpct	Human/mouse
SLC16A10/Slc16a10	None	MCT10/Mct10	TAT1/Tat1	Human/mouse
SLCO1C1/Slco1c1	*SLC21A14/Slc21a14*	OATP1C1/Oatp1c1	OATP-F/Oatp14	Human/mouse & rat
Slco1a4	*Slc21a5*	Oatp1a4	Oatp2	Mouse & rat
SLCO3A1/Slco3a1	*SLC21A11/Slc21a11*	OATP3A1/Oatp3a1	OATP-D/Oatp11	Human/mouse & rat
SLCO2B1	*SLC21A9/Slc21a9*	OATP2B1/Oatp2b1	OATP-B/Oatp9	Human/mouse & rat
SLCO4A1/SLCO4a1	*SLC21A12/Slc21a12*	OATP4A1/Oatp4a1	OATP-E/Oatp12	Human/mouse & rat
SLC7A5/Slc7a5	None	LAT1/Lat1	None	Human/mouse & rat
SLC7A8/Slc7a8	None	LAT2/Lat2	None	Human/mouse & rat
SLC10A1/Slc10a1	None	NTCP/Ntcp	GIG29/None	Human/mouse & rat

L-type amino acid transporters

The L-type amino acid transporters are heterodimeric proteins, with a 12-transmembrane domain light chain (SLC7) and a glycosylated heavy chain with a single transmembrane domain. These proteins transport neutral amino acids, such as leucine, phenylalanine and tyrosine. Large neutral amino acids transporter small subunit (commonly known as LAT1 and encoded by *SLC7A5*) and Large neutral amino acids transporter small subunit 2 (LAT2, encoded by *SLC7A8*) are sodium-independent transporters that, in addition to amino acids, transport T_4 and T_3, and have a K_m in the μM range (Table 1).[31,32] LAT1 is a more efficient T_3 transporter than LAT2.[33] *Slc7a5* and *Slc7a8* are widely expressed in the developing mouse brain (Figure 1).[21] *Slc7a5* is a prominent transporter in neurons, astrocytes, oligodendrocyte precursor cells and microglia, and is very abundant in endothelial cells of the microvasculature (that is, the blood–brain barrier).[34] In mice, *Slc7a8* is expressed in neurons, oligodendrocyte precursor cells, microglia and endothelial cells (Figure 1). *In situ* hybridization studies in mice indicate that *Slc7a8* mRNA is present in neurons across different regions of the brain.[35] This pattern seems to be complementary to that of *Slc16a2* mRNA (encoding Mct8).[28] In humans, LAT2 is localized to adult neurons and microglia, but is absent from fetal neurons.[25]

OATPs

OATPs are proteins with 12 transmembrane domains that mediate transport of amphipathic organic compounds, such as steroids, bile salts, drugs and anionic oligopeptides.[36,37] Up to seven human and 14 mouse proteins are active in thyroid hormone transport *in vitro* with K_m for T_4 and T_3 in the μM range (Table 1).[38,39] In transcriptomic data from mice, most genes of the *Slco* family encoding proteins with thyroid hormone transporter activity are expressed at very low levels in the postnatal brain.[21] Transporter genes expressed at notable levels are shown in Figure 1 and include *Slco1c1* (encoding Oatp1c1) and solute carrier organic anion transporter family members 1a4 (*Slco1a4,* encoding Oatp1a4), 3a1 (*Slco3a1*, encoding Oatp3a1) and 2b1 (*Slco2b1*, encoding Oatp2b1). Rat Oatp1a4 transports T_4 and T_3,[40,41] and the mouse gene *Slco1a4* is expressed almost exclusively in endothelial cells (Figure 1). Human OATP1C1 transports T_3, rT_3, T_4 and T_4 sulphate with high specificity.[40] This protein has the lowest K_m for T_4 among all transporters (0.09 μM).[42] Oatp1c1 is present in astrocytes and endothelial cells in the developing mouse brain.[26,43] Human OATP3A1 transports T_4, and the mouse gene *Slco3a1* is expressed mostly in cells of the oligodendrocyte lineage in the brain (Figure 1).[44] Rat Oatp2b1 transports T_4, and the mouse gene *Slco2b1* is abundantly expressed in endothelial cells and microglia.[21,45]

Pathogenic MCT8 mutations

The identification of pathogenic mutations in MCT8 indicates a pathophysiological role for thyroid hormone transport. Inactivating mutations of *SLC16A2* are present in a rare syndrome characterized by severe X-linked psychomotor retardation known as Allan–Herndon–Dudley syndrome, which was first described in 1944.[46] Patients with this disease have global developmental delay, profound intellectual disability (with an IQ <30), rudimentary communicative skills and lack of speech, as well as severe neuromotor impairment with central hypotonia, spastic paraplegia and dystonic movements. These patients are unable to sit or stand independently.[12,13,46–55] In 2004, the investigators of two separate studies described patients with similar clinical impairment and altered serum concentrations of thyroid hormones, that is, increased T_3, low T_4 and rT_3, with normal or slightly raised levels of TSH.[12,13] These hormonal changes indicated a defect in thyroid hormone metabolism, and led to the identification of mutations in the gene encoding MCT8 (Box 1). *SLC16A2* gene mutations were subsequently found in family members of the patients originally described as having Allan–Herndon–Dudley syndrome.[48]

Slc16a2 knock-out mice were generated as experimental models of Allan–Herndon–Dudley syndrome.[56,57] Unfortunately, the *Slc16a2* knock-out mice are not a good model of the neurological component of the syndrome. These mice have normal brain development and structure, with minimal alterations of behaviour.[25] However, these mice do faithfully reproduce the changes in the serum concentrations of T_4, T_3, rT_3 and TSH found in

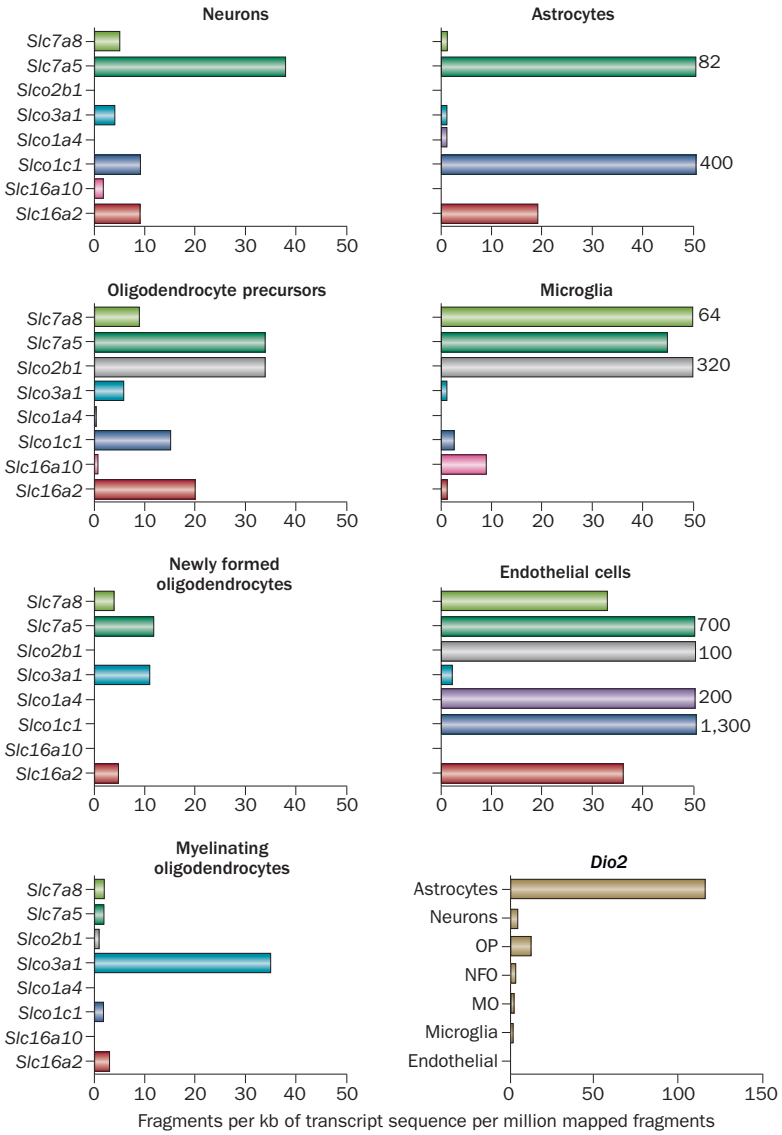

Figure 1 | Thyroid hormone transporter expression in the postnatal mouse brain. Original data were retrieved from a public database constructed from the transcriptomic analysis of purified native cell populations using RNAseq.[21] Data from the expression of each transporter among all cell types were reorganized to obtain the distribution of relevant transporters for each cell type. Oligodendrocyte precursors have 5% microglial contamination, which might have influenced their *Slco2b1* mRNA content.[21] The distribution of *Dio2* is shown for comparison. These data represent the relative abundance of RNA molecules expressed as RNA fragments per kb of transcript sequence per million mapped fragments. Values exceeding the upper limit of the scale are given in numbers. These data do not permit quantitative comparisons of mRNA abundance between different cells. Neurons, astrocytes and endothelial cells were isolated from the postnatal day 7 cerebral cortex. OP, NFO, MO and microglia were obtained from postnatal day 17 stage mice. Abbreviations: MO, mylelinating oligodendrocytes; NFO, newly formed oligodendrocytes; OP, oligodendrocyte precursors.

the human patients.[56,57] Consequently, in spite of being a partial model of the disease, these mice, and other models based on single or compound inactivation, have been useful for the analysis of altered serum concentrations of thyroid hormone, and to formulate hypotheses to explain differences in the neurological outcomes between humans and mice (Table 3).

Mechanisms of MCT8 deficiency

The sequence of events leading to the altered thyroid hormone concentrations in serum is likely to be similar between mice and humans, and results from mouse models can help to understand the endocrine component of MCT8 deficiency. However, the mechanisms are very complex, and incompletely understood. Most data have been obtained in mature mice, which represent the end result of a chain of interconnected events that might have begun in early embryonic development. Altered thyroid hormone secretion, transport and metabolism lead to defective mechanisms (both intrinsic to the thyroid and extrathyroidal) in several organs (Figure 2). Increased DIO1 activity has an essential role in the increase in serum levels of T_3, which is supported by the observation that in Mct8-deficient mice, the inactivation of this deiodinase normalizes circulating T_3 concentrations (Table 3).[58]

Thyroid gland

In the mouse and the human thyroid, MCT8 is expressed in the basolateral side of the membrane of thyroid follicular cells and mediates thyroid hormone efflux to the blood.[59–61] *Slc16a2* inactivation in mice leads to diminished secretion of T_4 and retention of T_4 and T_3 in the thyroid gland,[59,60] which contributes to the low serum levels of T_4. Whether T_3 secretion is increased or decreased is unclear and conflicting evidence has been presented. T_3 production might be increased at the expense of T_4 deiodination by deiodinases expressed in the thyroid gland.[60,62] In the absence of MCT8, T_3 might also exit the thyroid gland via another transporter, such as MCT10 and contribute to the increase in serum concentration of this thyroid hormone.[63] However, in a study on the dynamics of T_4 and T_3 release from the thyroid gland, performed by chemically blocking thyroidal secretion for a few days and then measuring serum levels of T_4 and T_3 at different times after eliminating the block, T_3 and T_4 secretion proceeds at a slower rate in the Mct8-deficient mice than in wild-type mice.[59] This result indicates that the secretion of T_3 is reduced in the same way as T_4 in Mct8 deficiency. Although Mct10 also seems to be active in thyroidal secretion, if this transporter permitted increased secretion of T_3, double knock-out mice for *Slc16a2* and *Slc16a10* (Table 3) should have decreased T_3 secretion and reduced levels of this hormone in the serum; however, this outcome is not the case.[63] A total thyroidectomy in a patient with defective MCT8 who received T_4 substitution therapy had a pattern of low serum levels of T_4 and high T_3,[61] which indicates that in humans, MCT8 deficiency does not increase thyroidal T_3 secretion.

Brain

The concentrations of T_4 and T_3 in the mouse brain are also reduced in response to Mct8 deficiency (Figure 2).[56,57] Thyroid hormone degradation by DIO3 is very active in the brain, especially during embryonic development, with the conversion of T_4 to rT_3 and T_3 to T_2.[64] A major consequence of Mct8 deficiency in mice is a block in T_3 entry in the brain,[56,57] which leads to diminished T_3 degradation. Brain uptake of T_4 is unaffected, but its supply is

Box 1 | Molecular genetics of MCT8 mutations

SLC16A2, which encodes monocarboxylate transporter 8 (MCT8), is located on the X chromosome (Xq13.2), consists of six coding exons and contains two translational start sites that potentially code for proteins of 613 and 539 amino acids, respectively.[104] To date, 76 mutations in *SLC16A2* have been reported, and are distributed throughout the six exons. These mutations include 11 gross deletions, 10 small deletions, 13 small insertions, two splice-site mutations, one complex rearrangement and 39 missense or nonsense mutations.[48,105–107]

Complete loss-of-function is expected from gross deletions and mutations that produce a truncated protein. Functional analysis of mutated proteins after transient expression in different cell types reveals that mutations might result in reduced amounts of cellular protein, impaired trafficking to the plasma membrane or a lack of thyroid hormones transport capacity. The mutant's activity depends on the cell type in which it is expressed,[108,109] and is probably due to expression of chaperone proteins responsible for the folding and trafficking to the membrane. So far, the only protein shown to bind and modify the subcellular location of MCT8 is the pituitary hormone transforming interacting protein.[110]

Differences in phenotype among patients with Allan–Herndon–Dudley syndrome might depend on the molecular properties of the mutated MCT8 protein, possible changes in modifier genes, or on the hypothetical transport of other substrates. Some point mutations, such as S194F (10 patients), L434W (nine patients), L492P (one patient), L568P (28 patients including the original family) and Phe501del (three patients), result in a fairly mild form of the disorder, with limited, dysarthric speech, and some walking capacity with ataxic gait. In general, the milder phenotypes show membrane localization of the mutated MCT8 with significant residual thyroid hormone transport capacity.[105,109]

reduced because the serum concentrations are lower than normal. This process activates DIO2, and conversion of T_4 to T_3 increases.[56,57] The reduced levels of T_4 as a substrate for DIO3 possibly results in reduced rT_3 formation, but this possibility needs to be confirmed in *Slc16a2* and *Dio3* double knock-out mice.

Liver and kidney

In the liver, levels of T_3 are raised and levels of T_4 are reduced,[56,58] and in the kidney, levels of both T_3 and T_4 are increased.[65] Transport of thyroid hormones to the liver and kidney in Mct8-deficient mice is not compromised due to the expression of alternative transporters, including Mct10.[63] Raised levels of T_3 activate DIO1 in both organs with increased production of T_3 at the expense of T_4.[56,57,65] Furthermore, rT_3 is also a substrate of DIO1, and the increased activity of this deiodinase increases rT_3 degradation. In the kidney, the absence of Mct8 decreases efflux of T_4 and T_3, which are then retained in the parenchyma.[65]

Muscle, adipose tissue and bone

Metabolism and consumption of glucose are increased in the skeletal muscle of Mct8-deficient mice, probably as a result of an increased tissue concentration of T_3.[66] The sustained hypermetabolism leads to muscle consumption. However, in brown adipose tissue, T_3 levels are normal.[66] The brown adipocytes respond to the low serum levels of T_4 with increased DIO2 activity, which is similar to the response in the brain. This mechanism compensates for the decreased thyroid hormone uptake due to lack of Mct8,[66] and leads to normal brown adipose tissue function. Few data on the role of specific transporters in the bone tissue exist; however, in chondrocytes, Mct10 seems to function as a physiological thyroid hormone transporter.[67]

MCT8 deficiency in the perinatal period

Overall, in MCT8-deficient organisms, signs of thyroid hormone excess (in the liver, kidney and muscle), and deprivation (the brain) are apparent. T_4 is reduced and T_3 increased in the serum due to a combination of altered thyroidal secretion and altered tissue metabolism. To achieve the final adult pattern, the changes in circulating thyroid hormones in Mct8-deficient mice go through different phases that deviate from those of normal mice. The first event is an increase in serum levels of T_4 by embryonic day 18, which persists during the perinatal period.[35,68] T_4 then decreases by postnatal day 7 and is already low by postnatal day 21. Changes in circulating levels of T_3 are unclear, but raised levels of T_3 do not seem to occur until at least postnatal day 5.[35] Interestingly, the perinatal period in Mct8-deficient mice is characterized by increased brain levels of T_3 and increased expression of genes regulated by thyroid hormone.[35,68] The mechanisms underpinning these changes are not well understood, and changes in placental transport might also influence thyroid hormone levels.[68] One factor might be that the restriction in DIO3-mediated thyroid hormone degradation, which is very active during the fetal and early postnatal periods,[69] leads to a redistribution of T_4 and T_3 depending on the developmental expression patterns of secondary transporters.

TRH–TSH in MCT8 deficiency

Patients who have an MCT8 deficiency usually have slightly raised levels of TSH, which in the presence of increased T_3, indicates a partial resistance of the thyrotropin-releasing hormone (TRH)–TSH axis to thyroid hormones.[70] Although the TRH stimulation test has been performed in only a few patients, the response is usually normal; however, one patient had a blunted response that was possibly due to very increased levels of T_3.[71] Mct8-deficient mice also have moderately increased basal levels of TSH.[56,72] Furthermore, when the mice are rendered hypothyroid, 2.5 times more T_4 and sixfold more T_3 are required for TSH normalization compared with hypothyroid wild-type mice.[56,72] *Slc16a2* inactivation leads to an increased expression of TRH in the hypothalamic paraventricular nucleus,[57] which indicates that Mct8 in the membrane of the TRH neurons is important for T_3 action in these cells. Tanycytes are a specific type of glial cells that line the walls of the third ventricle with processes in contact with blood vessels and the cerebrospinal fluid. These cells express *Dio2*, *Slc16a2* and *Slco1c1* and deliver T_3 to the hypothalamic nuclei,[73] but whether these genes influence the effects of MCT8 deficiency is unknown. In humans, the role of MCT8 in the regulation of TSH secretion at the pituitary level is unclear. In the adult pituitary gland, MCT8 and DIO2 are not present in the thyrotrophs, but are found in subsets of folliculo-stellate cells.[74] MCT8 has been proposed to be involved in the efflux of the T_3 formed after T_4 deiodination.[74]

Table 3 | Phenotype of mouse models of transporter deficiency

Transporter deficiency	Thyroid hormone, TSH and deiodinase levels					Brain phenotype
	Serum	Thyroid	Liver	Kidney	Brain	
Mct8[25,56–60,76,79,92]	T_3 ↑↑ T_4 ↓↓ rT_3 ↓↓ TSH ↑	T_3 ↑↑ T_4 ↑↑ DIO1 ↑↑	T_3 ↑↑ T_4 ↓ DIO1 ↑↑	T_3 ↑↑ T_4 ↑↑ DIO1 ↑↑	T_3 ↓↓ T_4 ↓↓ DIO2 ↑↑↑ DIO3 ↓↓	Impaired T_3 transport, normal T_4 transport Gene expression: changes depend on developmental stage* TRH expression ↑, TSH ↑, normal motor function, increased metabolic activity, decreased anxiety-like behaviour, mild hyperalgesia
Mct8-Dio2[58,79,64]	T_3 ↑↑ T_4 ↓↓ TSH ↑↑↑ rT_3 ↓↓↓	T_3 ↑↑ T_4 ↑↑	T_3 ↑↑ T_4 ↓↓ DIO1 ↑↑	ND	T_3 ↓↓ DIO3 ↓↓	Gene expression: mostly hypothyroid Increased metabolic activity
Mct8-Dio1[58]	T_3 norm T_4 ↑ TSH ↑ rT_3 norm	T_3 ↑↑ T_4 ↑↑	T_3 norm T_4 norm	ND	T_3 norm DIO2 ↑↑↑ DIO3 ↓	Gene expression: mildly hypothyroid
Mct8-Oatp1c1[80]	T_3 ↑↑ T_4 ↓↓ TSH ↑↑↑	ND	DIO1 ↑↑	DIO1 ↑↑	T_3 ↓↓↓ T_4 ↓↓↓ DIO2 ↑↑↑ DIO3 ↓	Impaired T_4 transport Gene expression: hypothyroid TRH expression norm, TSH norm, impaired motor function, abnormal cerebellar histology, hypomyelination
Oatp1c1[80,81]	T_3 norm T_4 norm TSH norm	ND	DIO1 norm	DIO1 norm	T_3 norm T_4 ↓↓ DIO2 ↑↑ DIO3 norm	Impaired T_4 transport Gene expression: mildly hypothyroid Normal motor function Mostly normal brain histology
Mct8-Lat2[35]	T_3 ↑ T_4 ↓↓	ND	DIO1 ↑↑↑	ND	T_3 ↓↓	Gene expression: norm in adults and neonates
Lat2[35,84]	T_3 ↓ T_4 ↓ TSH norm	ND	DIO1 norm	DIO1 norm	T_3 ↓	Gene expression: norm Slightly abnormal motor coordination Normal cerebellar histology
Mct8-Mct10[63]	T_3 ↑↑ T_4 norm TSH ↑	T_3 ↑↑↑ T_4 ↑↑↑	T_3 ↑↑↑ T_4 ↑↑ DIO1 ↑↑↑	T_3 ↑↑↑ T_4 ↑↑ DIO1 ↑↑↑	T_3 ↓ T_4 ↓	Gene expression: changes depend on developmental stage* TRH expression norm, TSH ↑
Mct10[63]	T_3 ↓ T_4 norm TSH ↓↓	T_3 norm T_4 norm	T_3 norm T_4 norm DIO1 norm	T_3 norm T_4 norm DIO1 norm	T_3 norm T_4 norm	Gene expression: norm TRH expression norm, TSH norm

Endocrine and neurological phenotypes of several mice models from postnatal week 3 onwards. Brain data correspond to cerebral cortex and/or striatum. Comparisons are with control animals: (↑) slightly high, (↑↑) high, (↑↑↑) very high, (↓) slightly low, (↓↓) low, (↓↓↓) very low. *Mct8-deficient mice have mostly normal gene expression with some exceptions (*Hr, Nrgn*). Neonates have increased gene expression of genes positively regulated by T_3 (*Hr, Klf9, Shh*), and decreased expression of genes negatively regulated by T_3 (*Aldh1a3*), in the cerebrum and cerebral cortex, indicating cerebral hyperthyroidism. Lat2 deficiency prevents the Mct8-induced cerebral hyperthyroidism. Abbreviations DIO1, type 1 deiodinase; DIO2, type 2 deiodinase; DIO3, type 3 deiodinase; KO, knock-out; ND, not determined; norm, normal.

The blood–brain barrier

The blood–brain barrier is formed primarily by the microvascular endothelial cells, joined by tight junctions that prevent paracellular transport. Carrier-mediated transport of thyroid hormones through the blood–brain barrier was first suggested in the 1970s.[75] The importance of MCT8 at the blood–brain barrier is supported by kinetic, functional and anatomic evidence. The Mct8-deficient mouse brain has a selective defect in T_3 uptake, whereas T_4 accumulation is normal.[56,57] When these hormones were administered separately to hypothyroid control mice, both induce changes in the expression of *Hr* and *Nrgn*, two well-known T_3 target genes.[76] By contrast, after administration to hypothyroid Mct8-deficient mice, T_3 was inactive but T_4 was active.[76] T_3 acts directly on the nuclear thyroid receptor;[1] however, T_4 has low affinity for this receptor[77] and its effect on gene expression at physiological concentrations is largely due to conversion to T_3. In the brain, this pathway takes place in the astrocytes and is mediated by DIO2 (Figures 1 and 3);[78] DIO2 activity is increased in conditions of Mct8 deficiency.[56,57] These experiments indicate that the normal transport of T_4 in the Mct8-deficient mouse brain efficiently compensates for the impaired T_3 transport. This result might explain the lack of a neurological phenotype in these mice and the normal expression of most T_3-regulated genes in the Mct8-deficient mouse brain.[79]

MCT8 is present in microvessels of the rodent and human brain.[26] In the Mct8-deficient mouse, T_4 is still transported to the brain, as the brain microvessels also express the T_4 transporter gene *Slco1c1* (Figures 1 and 3).[26] Mice deficient in Mct8 and Oatp1c1 have diminished brain uptake and brain concentrations of T_4 and T_3, impaired motor function, as well as hypothyroid-like gene expression patterns and cerebellar histology (Table 1).[80] In contrast to rodents,[81] OATP1C1 is present

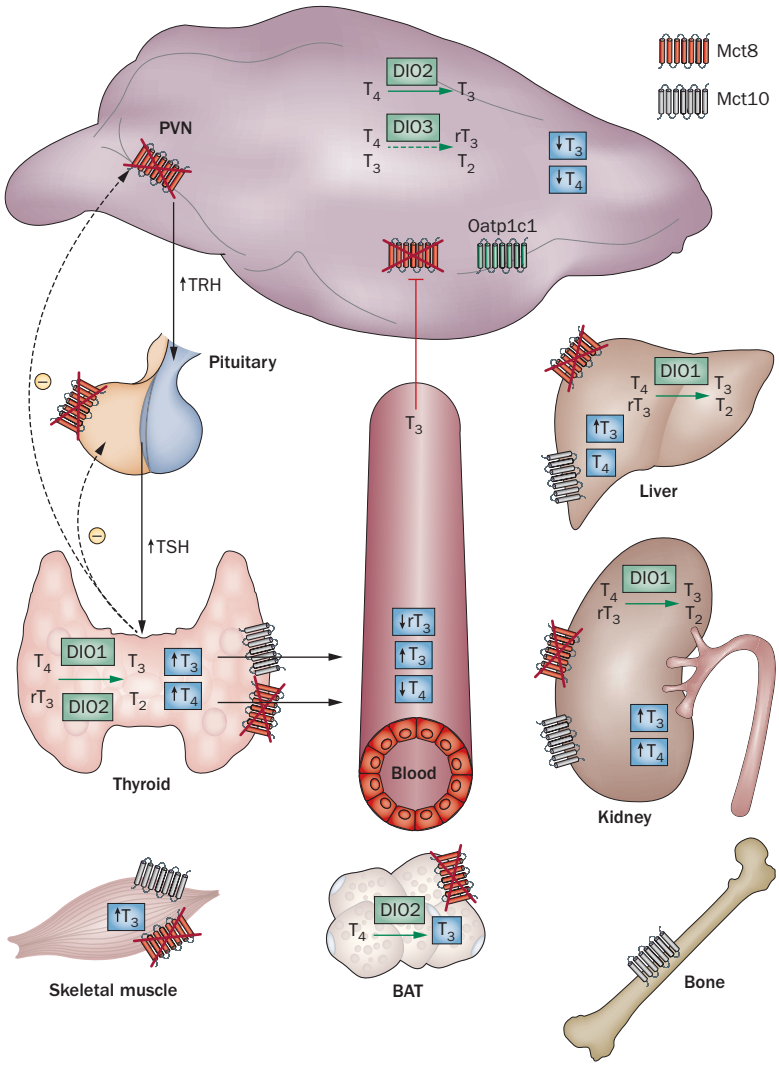

Figure 2 | Changes in thyroid hormone concentration in Mct8 knock-out mice. In the serum, T_4 and rT_3 are low and T_3 is raised. In the thyroid gland, Mct8-deficiency leads to reduced secretion and increased retention of T_4 and T_3. T_3 and T_4 are also increased by deiodination. Mct10 is an alternative thyroid hormone transporter. In the hypothalamic–pituitary axis, deficient thyroid hormone entry into the hypothalamic PVN and the pituitary impairs a negative feedback loop with raised levels of TRH and resistance of the TRH–TSH axis to thyroid hormone. In the brain, deficient T_3 entry reduces deiodination by DIO3 and contributes to the increased levels of T_3 in serum. T_4 supply is reduced, but transport through Oatp1c1 permits T_4 to T_3 conversion via increased DIO2 activity. In the liver, increased supply of T_3 from the serum produces liver hyperthyroidism, with increased DIO1 activity, production of more T_3 from T_4, and increased degradation of T_4 to rT_3. In the kidney, a lack of Mct8 decreases efflux with retention of T_4 and T_3 and thyrotoxic changes, including increased DIO1 activity as in liver. In bone, Mct10 might mediate T_3 transport and action in chondrocytes. In BAT, reduced T_4 levels lead to increased DIO2 activity with increased formation of T_3, which results in normal T_3 concentrations and BAT function. In skeletal muscle, the increased T_3 content raises energy expenditure. Abbreviations: BAT, brown adipose tissue; DIO1, type 1 deiodinase; DIO2, type 2 deiodinase; DIO3, type 3 deiodinase; PVN, paraventricular nucleus; TRH, thyrotropin-releasing hormone.

at very low concentrations in the human and monkey brain,[26,82] which suggests that loss-of-function mutations in MCT8 in these species cannot be compensated for by selective T_4 transport and local conversion to T_3 as occurs in mice. Differences between the neurological phenotype between mice and patients with MCT8 mutations can, therefore, be explained by differences in expression of the T_4 transporter OATP1C1 in the blood–brain barrier. Additionally, phenotypic differences might exist from the contribution of OATP1C1 to thyroid hormone uptake in other cells of the central nervous system.

Role of other transporters

Other genes that encode proteins with the capacity to transport T_4 are also expressed in the blood–brain barrier in rodents, such as *Slco1a4* (Figure 1).[83] The encoded protein, Oatp1a4, has no human homologue. However, OATP1A2 is a related protein, with ~70% homology,[83] that is present in the blood–brain barrier of monkeys[82] but at a concentration 34% lower than Oatp1a4 in the mouse. Nevertheless, current evidence suggests these proteins are not involved in T_4 transport. For example, a combined Mct8 and Oatp1c1 deficiency in mice results in almost complete thyroid hormone deprivation in the brain,[80] which suggests that no other transporters expressed in the blood–brain barrier can sufficiently compensate for the double Mct8 and Oatp1c1 deficiency. In the mouse, expression of a few T_3-regulated genes, such as *Hr*, *Cbr2* and *Nrgn*, are decreased, whereas expression of *Dgkg* is increased by the lack of Mct8.[57,58,76,80] However, these changes are not as great as those seen in hypothyroidism, and most mice maintain normal gene expression[79] due to enhanced local T_3 production from T_4. Consequently, even if Mct8 is absent from the neuronal membrane, T_3 generated in the astrocytes might enter these and other neural target cells through alternative transporters (Figures 1 and 3). However, the data to support a role of any specific transporter are very limited. Lat2 might have a role restricted to the perinatal period. As noted previously in this Review, Mct8-deficient mice present with cerebral hyperthyroidism during the perinatal period;[35,68] concomitant Lat2 deficiency prevents cerebral hyperthyroidism.[35] This result might indicate a possible role for Lat2 in T_3 delivery that is limited to the perinatal period, but the available data do not permit formulating a solid mechanistic hypothesis of Lat2 function.[35,84]

Transport in the choroid plexus

MCT8 and OATP1C1 are also present in the choroid plexus of rats,[85] mice,[26] chickens[86] and humans,[26] but their role in thyroid hormone transport to the parenchyma through the choroid plexus is unclear (Figure 3). In adult rats, T_3 delivered into the ventricular system penetrated only a few millimetres into the brain parenchyma, and remained in a periventricular location.[87] This finding supports the view that transport through the choroid plexus is less relevant than transport through the blood–brain barrier. This situation might be different during fetal and early postnatal life, as the size of the ventricular system and relative volume of the cerebrospinal fluid with respect to the parenchyma are higher than in adult animals.[88] The intense immunofluorescence signals for Oatp1c1 and Mct8 proteins in the choroid plexus during fetal life, which is much higher than in the blood–brain

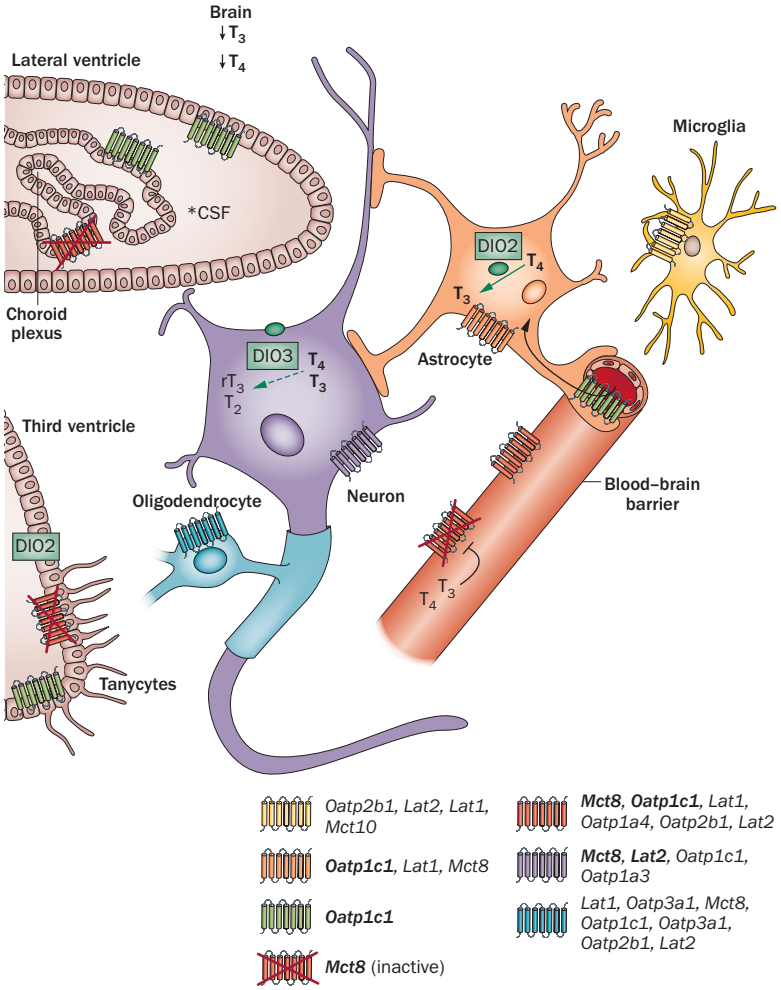

Figure 3 | Thyroid hormone transport in the mouse brain. Transporters for which there is experimental evidence for function are shown in bold. In the blood–brain barrier, endothelial cells contain Mct8 and Oatp1c1. In the absence of Mct8, T_3 transport is compromised, but T_4 is still transported via Oatp1c1, a transporter that is also present in the astrocyte endfeet in close contact with the endothelial cells. T_4 in the astrocytes acts as a substrate for DIO2, which provides the brain with enough T_3 to compensate for the lack of T_3 transport. The transporter that mediates T_3 efflux from the astrocytes has not been identified. T_4 and T_3 degradation takes place in neurons by DIO3, located in the membrane. DIO3 activity is low in Mct8 deficiency due to the reduced supply of T_3. The tanycytes express Mct8 and Oatp1c1, and also DIO2, and might be involved in the production of T_3 and its supply to hypothalamic nuclei. Oatp1c1 and Mct8 are also present in the epithelial cells of the choroid plexus, and Oatp1c1 is also present in the ependymocytes. *T_4 is low in the CSF in MCT8 deficient patients, and T_3 has been reported to be normal.[54] Abbreviations: CSF, cerebrospinal fluid; DIO2, type 2 deiodinase; DIO3, type 3 deiodinase.

barrier or the adjacent parenchyma, suggests an important role for these proteins in thyroid hormone transport.[85] However, the pathophysiological importance of T_4 and T_3 in the cerebrospinal fluid remains uncertain. In euthyroid patients, free T_4 and T_3 concentrations in the cerebrospinal fluid (77 pM and 0.2 pM, respectively) were of the same order as in serum (28 pM and 0.3 pM, respectively).[89] In one MCT8-deficient patient, free T_4 in the cerebrospinal fluid was reduced to half of normal levels (1.3 pM versus 2.5 pM), whereas free T_3 was equal to normal (0.7 pM) despite the increased serum levels of T_3.[54] Given the paucity of data, the pathophysiological importance of thyroid hormones in the cerebrospinal fluid is unknown.

The brain in MCT8 deficiency

The marked hypotonia and muscle weakness present in infants and young children with Allan–Herndon–Dudley syndrome progressively develops into spasticity and spastic quadriplegia.[47,49] Patients have profound cognitive impairment, lack of speech, athetoid movements and dyskinesia.[90,91] These symptoms indicate damage to the cerebral cortex and basal ganglia, as well as to the pyramidal and extrapyramidal systems.

Mice

In mice, Mct8 deficiency leads to only minor alterations of behaviour, and the brain structure is normal.[25] Metabolic studies using ^{13}C NMR spectroscopy in 7-month-old mice, reveal increased metabolic activity, with increased flux through the tricarboxylic acid cycle and enhanced GABAergic neurotransmission.[92] However, these findings could not be correlated with hypothyroidism or hyperthyroidism, and were interpreted as the result of long-term compensation of some early alterations that were induced by Mct8 inactivation. The combined Mct8 and Oatp1c1 deficiency in mice results in a state of typical brain hypothyroidism.[80] However, whether these mice are a model of the human MCT8 deficiency, or just another model of hypothyroidism is still unclear.

Zebrafish

MCT8-deficient zebrafish are an emerging model of the neuropathological mechanisms in MCT8 deficiency. The zebrafish *slc16a2* gene shares 56% identity[93] with its mammalian orthologues, and is expressed in blood vessels, neurons and possibly oligodendrocyte precursor cells.[94] MCT8-deficient zebrafish have altered development of the nervous system, with reduced axonal branching and synaptic density, and deficient myelination with 30% decreased expression of the myelin gene *p0*.[95] They also have impaired motor and sleep behaviour, and altered responses to sensory stimuli.[95,96] Neurological and behavioural deficiencies appear very early in development, at 3 days after fertilization, without changes in the thyroid-hormone-dependent genes *klf9* and *nrgna*. On the basis of these data, the investigators proposed a thyroid-hormone-independent action of mct8 at very early stages of development.[95] If confirmed in other animal models, this phenomenon might provide additional insights into the human disease. Interestingly, the thyroid hormone analogues triac, tetrac (3,5,3',5'-tetraiodo-thyroacetic acid) and ditpa (3,5-diiodo-thyropropionic acid) can normalize expression levels of *p0* and partially rescued the neurological phenotype.[95] The zebrafish system, therefore, seems ideally suited for high-throughput screening of therapeutic drugs in MCT8 deficiency.

Humans

Analysis of the brain of MCT8-deficient patients agrees with the concept that the brain damage is due to deficient

Box 2 | *MCT8* mutations and cretinism

Thyroid hormone deficiency during fetal and postnatal development might lead to profound neurological and cognitive impairment. Specifically, the severe iodine deficiency in endemic cretinism might be associated with intellectual impairment, deaf-mutism, and a neuromotor disorder with truncal and proximal limb rigidity and spasticity, a condition known as neurological cretinism. Pyramidal and extrapyramidal signs might also be present. Neurological impairment indicates irreversible damage to the cochlea, the cerebral cortex and basal ganglia.[111–113] Damage to the brain arises from severe maternal hypothyroxinaemia and profound iodine deficiency during fetal life, especially in the second trimester, during which expression of the thyroid hormone receptors increases in brain.[114] While this condition has some resemblance to the neurological involvement in MCT8 as observed in adults with MCT8 mutations, the clinical picture is clearly different, which should lead to a search for MCT8 actions independent of thyroid hormone transport. Investigators using a zebrafish model of MCT8 deficiency indicate that this protein might have thyroid-hormone-independent actions during early embryonic development.[95] However, the clinical relevance of the perinatal cerebral hyperthyroidism observed in Mct8-deficient mice is unknown.[35,68] Nevertheless, comparisons between endemic cretinism and MCT8 deficiency are difficult, because the former has many clinical forms that depend on different factors in addition to iodine deficiency, and which also depend on the geographical setting. Unfortunately, comparing thyroid hormone deficiency that affects the whole body with the selective deprivation of the brain in MCT8 deficiency, which might have time and location-dependent expression of thyroid hormone transporters, is not possible. Consequently, while the possibility of thyroid-hormone-independent actions of MCT8 remain possible, phenotypic differences between neurological cretinism and MCT8 deficiency cannot be taken as an *a priori* support for this hypothesis.

thyroid hormone transport (Box 2).[97] Accordingly, concentrations of T_4, T_3 and rT_3 were reduced by ~50% in the fetal brain of an affected fetus, although more drastic reductions could perhaps have been expected given the severity of the neurological impairment. Changes in deiodinase expression, with increased *DIO2* expression and DIO2 activity, and decreased *DIO3* expression, were also compatible with hypothyroidism, as were the specific histopathological alterations.[97]

Qualitatively similar signs of tissue damage were found in a 30-week-old fetus and an 11-year-old child who had an MCT8 deficiency.[97] Findings included immaturity and deficient development of the cerebral cortex and cerebellum, delayed myelination in the fetus and hypomyelination in the 11-year-old child (Figure 4). Altered expression of calcium-binding proteins with lack of parvalbumin-expressing interneurons in the cerebral cortex, as well as signs of deficient neuronal differentiation with altered expression of neurofilaments and the synaptic protein synaptophysin, were also present (Figure 4).[97] These defects are also observed in models of thyroid hormone deficiency in rodents.[98–100] Deficient myelination is often seen using MRI in some patients with MCT8-deficiency.[55,90,101–103] Defective myelination is observed in young patients, and might no longer be evident beyond 5–6 years of age using currently available MRI techniques.[90,102,103] Consequently, the defect is often interpreted as a delay in myelination.[90,102,103] The persistence of the myelination defect in the 11-year-old child as observed with myelin basic protein immunostaining, indicates that this defect might persist beyond childhood and is a true hypomyelination. The difference between permanent hypomyelination and delayed myelination is important because the latter might be a nonspecific feature of delayed development.

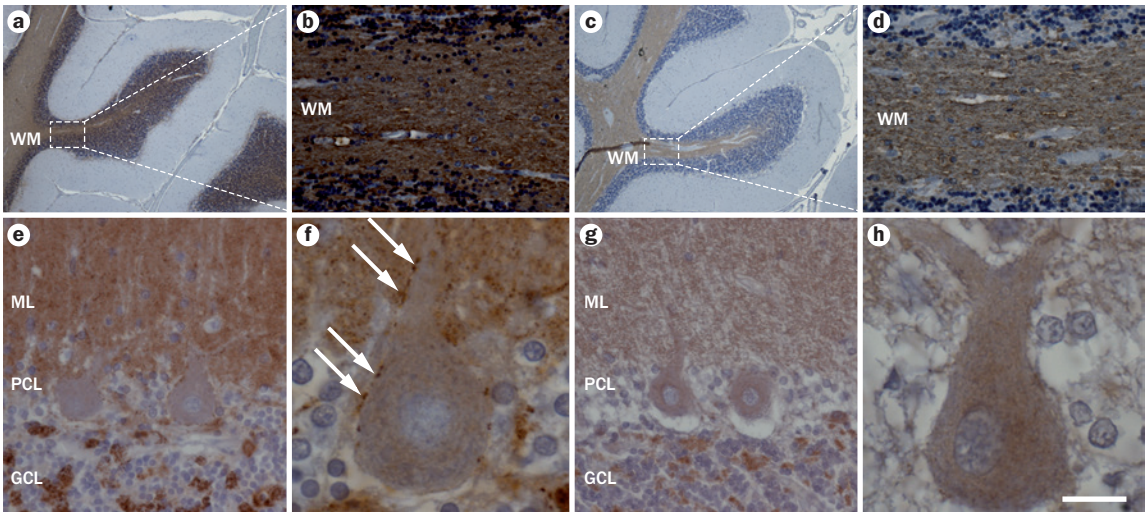

Figure 4 | Histopathology of MCT8 deficiency in humans. **a** | A cerebellar section immunostained with myelin basic protein and haematoxylin from a healthy 10-year-old child. **b** | An enlargement of the WM in panel a. **c** | A cerebellar section from an MCT8-deficient 11-year-old child immunostained with myelin basic protein and haematoxylin. **d** | An enlargement of the WM in panel c. **e** | A cerebellar section immunostained with synaptophysin and haematoxylin from a healthy 10-year-old child. **f** | An enlargement of a single Purkinje cell stained with synaptophysin and haematoxylin from a healthy 10-year-old child. Synaptophysin appears as dark puncta on the cell surface (arrows). **g** | A cerebellar section immunostained with synaptophysin and haematoxylin from the MCT8-deficient 11-year-old child. **h** | No synaptophysin puncta are observed in the Purkinje cells of the MCT8-deficient 11-year-old child. The MCT8-deficient sections have reduced levels of myelin basic protein, which indicates deficient myelination, and reduced synaptophysin content, which reveals a profound synaptic defect. Scale bar represents 540 μm (panels a, c); 60 μm (panels b, d); 66 μm (panels e, g); 26 μm (panels f, h). Abbreviations: ML, molecular layer; PCL, Purkinje cells layer; GCL, granular cell layer; WM, white matter. Permission obtained Endocrine Society © López-Espíndola, D. *et al. J. Clin. Endocrinol. Metab.* **99**, E2799–E2804 (2014).

Conclusions

Hormone transport through the cellular membranes is a key process in thyroid hormone metabolism and action. Among the many proteins capable of transporting thyroid hormones, only MCT8 is a specific thyroid hormone transporter, and is of special relevance in the supply of thyroid hormones to the brain through the blood–brain barrier. Mutations in the gene encoding MCT8 lead to a severe X-linked neurodevelopmental syndrome with altered thyroid hormone secretion and metabolism. Mouse models of Mct8 inactivation do not reproduce the neurological picture of the patients but have been useful in the analysis of altered thyroid hormone metabolism, and the role of other transporters, such as Oatp1c1 and Mct10. Histopathological studies of brains from MCT8-deficient individuals show structural defects consistent with severe thyroid hormone deficiency. Future work should define the role of MCT8 in the human brain during embryonic development in greater detail, including the consequences of its disruption, and determine the real physiological role of so-called secondary thyroid hormone transporters.

Review criteria

A search for original articles published in English until October 2014 was performed using PubMed with following search terms: "thyroid hormone transporter" and "MCT8". The search terms "monocarboxylate transporter", "organic anion transporter", "OATP1C1", "OATP14", "MCT10" and "LAT2" were also used alone or in combination with "thyroid hormone". The reference lists of identified papers were also used to identify additional material.

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Author contributions

All authors researched data for the article, discussed the content, and reviewed and edited the manuscript before submission. J.B. wrote the manuscript.